PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ANTI-ULCER ACTIVITY OF EXCOECARIA AGALLOCHA LINN.

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In

PHARMACOLOGY

Submitted by

Reg. No.261225708

Under the Guidance of

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This is to certify that the dissertation entitled "***PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ANTI-ULCER ACTIVITY OF THE LEAVES OF EXCOECARIA AGALLOCHA LINN.*** Submitted by **Reg. No. 261225708** in partial fulfillment of the requirements for the award of the **Degree of Master of Pharmacy in Pharmacology** by **The Tamil Nadu Dr. M.G.R. Medical University,** Chennai is a bonafied record of work carried out by him in the department of pharmacology. JKK Munirajah Medical Research Foundation College of Pharmacy,Namakkal. During the academic year 2013-2014.

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DECLARATION

I hereby declare that the dissertation work entitled "***PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ANTI-ULCER ACTIVITY OF THE LEAVES OF EXCOECARIA AGALLOCHA LINN.,*** is based on the original work carried out by me under the guidance and supervision of **Mr. A.SURESH., M.Pharm., (Ph.D).,** for submission to The Tamilnadu Dr. M.G.R. Medical University, Chennai in the partial fulfillment for the **Degree of MASTER OF PHARMACY in Pharmacology**. This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other university. The information furnished in this dissertation is genuine to the best of my knowledge and belief.

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Parents

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INTRODUCTION

INTRODUCTION TO HERBAL MEDICINE

History of herbal medicine

Herbal medicine, sometimes referred to as botanical medicine or herbalism, involves the use of plants, or parts of plants, to treat injuries or illnesses. This field also covers the use of herbs or botanicals to improve overall health and wellness. Herbalist, herbal medicine practitioners, traditional medicine practitioners, and Ayurvedic, homeopathic, and naturopathic healers all use herbal remedies in their practices.(A.N.Kalia.,2009)

Medicine of plant origin is based upon the premise that plants contain natural substances that can promote health and alleviate illness(Akhtar AH.,1995). Seeds, leaves, stems, bark, roots, flowers, and extracts of all of these have been used in herbal medicine over the millennia of their use. These supplemental treatments have been delivered raw, in teas and tinctures, as topical applications, in liquid forms, and in pills and capsules. In the beginning the plants were consumed raw or combined with hot water as a soup or tea. Later, the plants were dried and crushed for other uses. The plants were found in the wild and uses were often based on superstitious or visual cues. As science began to take a closer look at herbal remedies, their use became more refined. Herbs, and other plants, are actually the precursors to many of today's medicinal drugs.

Today, many modern, and Western, medicine practitioners are beginning to look at herbal remedies for some common and not-so common, disorders. The lower

cost, and often safer use, has attracted many medical professionals. Some physicians use herbs to off-set the side effects of pharmaceuticals.

Importance of herbal therapies

Herbal medicines are prepared from a variety of plant material such as leaves, stems, roots, bark, etc. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments.

Herbal remedies can also be purchased in the form of pills, capsules or powders, or in more concentrated liquid forms called extracts and tinctures. They can apply topically in creams or ointments, soaked into cloths and used as compresses, or applied directly to the skin as poultices. The medicines of plant origin are more popular because of their less adverse effect.(Rajkapoor B.,2002)

Plants are considered to be medicinal if they possess pharmacological activities of possible therapeutic use. These activities are often known as a result of millennia of trial and error, but they have to carefully investigate if we wish to develop new drugs that meet the criteria of modern treatment.

The identification of the active principles in medicinal plants and investigation of the extract in order to ensure that they are safe, effective and has constant activity. The isolation of these active principles and determination of their structure, in order that they may be synthesized, structurally modified, or simply extracted more efficiently.

Prospects of Herbal Research

There is a worldwide green revolution**, (Mukherjee P.K2002).,** which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Furthermore, underlying this upsurge of interest in plants is the fact that many important drugs in use today were derived from plants or from starting molecules of plant origin.

ANATOMY AND PHYSIOLOGY OF STOMACH

The stomach is a hollow, muscular organ of the gastrointestinal tract between the oesophagus and the small intestine. The gastrointestinal tract is one of the major exocrine systems in the body. The stomach, a J – shaped organ is a temporary "storage tank" where chemical breakdown of protein begins and food is converted in to chyme. The stomach lies in the left hypochondriac, epigastric and umbilical regions of the abdomen. The adult stomach varies from 15-25 cm long, but its diameter and volume depend on amount of food it contains. The stomach is continuous with the oesophagus at the cardiac sphincter and with duodenum at the pyloric sphincter. The stomach is divided in to three regions: the fundus, the body and the antrum.(Elaine Ross & Wilson 2006; Sherwood 6th Ed(2001); Tortora G.J.2006).

The stomach mucosa and gastric glands

The gastric mucosa is the mucous membrane layer of the stomach which contains the glands and the gastric pits In men it is about 1 mm thick and its surface is smooth, soft, and velvety. It consists of epithelium, lamina propria, and the muscularis mucosa. Gastric glands are simple or branched tubular glands that

emerge on the deeper part of the gastric foveola, inside the gastric areas and outlined by the folds of the mucosa.

There are three types of glands: cardiac glands, oxyntic glands, and pyloric glands. The cardiac glands mainly contain mucus producing cells. Parietal cells, which secrete hydrochloric acid are scattered in the glands, with most of them in the middle part. The bottom part of the oxyntic glands is dominated by zymogen cells that produce pepsinogen. The pyloric glands contain mucus-secreting cells. Several types of endocrine cells are found in all regions of the gastric mucosa. In the pyloric glands contain gastrin producing cells; this hormone stimulates acid production from the parietal cells. ECL (enterochromaffine-like) cells, found in the oxyntic glands release histamine, which also is a powerful stimulant of the acid secretion.

The α-cells produce glucagon, which mobilizes the hepatic glycogen, and the enterochromaffin cells that produce serotonin, which stimulates the contraction of the smooth muscles.

Table 1.1: Gastric enzymes and their functions

Gastrin The hormone gastrin causes an increase in the secretion of HCl, pepsinogen and intrinsic factor from parietal cells in the stomach. It also causes	

increased motility in the stomach. Gastrin is released by G-cells in the stomach to distenstion of the antrum, and digestive products. It is inhibited by a pH normally less than 4 (high acid), as well as the hormone somatostatin.	

Cholecystokinin	Cholecystokinin (CCK) has most effect on the gall bladder, but it also decreases gastric emptying and increases release of pancreatic juice which is alkaline and neutralizes the chyme.
Secretin	In a different and rare manner, secretin, produced in the small intestine, has most effects on the pancreas, but will also diminish acid secretion in the stomach.
Gastric inhibitory peptide	Gastric inhibitory peptide (GIP) decreases both gastric acid and motility.
Enteroglucagon	enteroglucagon decreases both gastric acid and motility.

Gastric juice and its functions

The cells of the gastric mucosa secrete a fluid called gastric juice. About 2-3 litres of gastric juice are secreted daily by specialized glands in the mucosa.

Mucus: Prevents mechanical injury and chemical injury to the stomach by lubricating the contents and by acting as a barrier between stomach and corrosive gastric gland.

Hydrochloric acid: Denatures the protein present in food partially, acidify the food and stops the action of salivary amylase, kills ingested microbes, provides acid environment needed for digestion of pepsinogen.

Pepsin: It is the only proteolytic enzyme in the stomach. Pepsin is secreted in the inactive form called pepsinogen in the stomach. Pepsinogen converted into pepsin by hydrochloric acid and by pepsin already present in the stomach. They begin the digestion of protein by breaking them in to smaller fragments. It acts at pH 1.5-3.5.

Intrinsic factor: necessary for absorption of vitamin B_{12}.

Mineral salts and water: liquefies the swallowed food.

Gastric lipase: splits the short chain triglycerides in fat molecules in to fatty acid and monoglycerides. Stomach also secretes gastrin in to blood

Physiology of gastric secretion

Gastric acid secretion is a complex, continuous process in which multiple central and peripheral factors contribute to a common endpoint: the secretion of H^+ by parietal cells. The regulation of acid secretion by parietal cells is especially important in peptic ulcer and constitutes a particular target for drug action. The secretion of the parietal cells is an isotonic solution of hydrochloric acid (150 mMol/L).

The Cl^- is actively transported into canaliculi in the cell which communicates with the lumen of the gastric glands and thus with lumen. The Cl^- secretion is accompanied by K^+, which is then exchanged for H^+ from within the cell by H^+, K^+ - ATPase. Carbonic anhydrase catalyses the combination of carbon dioxide and water to give carbonic acid (HCO_3^-), which dissociate into H^+ and bicarbonate ions. **(Thompson *et al., 1983)***

$$H_2O + CO_2 \xrightarrow{\text{carbonic anhydrase}} H^+ + HCO_3^-$$

Secretion of gastric acid is accomplished by activity of an ion motive ATPase that exchanges cytosolic H^+ for luminal K^+, resulting in acidification of extracellular surface of parietal cell and secretion of gastric juice. Parietal cell H^+, K^+ - ATPase, often designated the gastric "proton pump", derives its energy for

transport of H^+ and K^+ from the hydrolysis of adenosine tri phosphate (ATP). In the parietal cell, the enzyme has access to the canalicular membrane that provides pathway for inward K^+ transport in exchanging for outer H^+ transport. The transport activity of H^+, K^+ - ATPase is regulated by levels of intracellular second messengers, either cAMP or calcium ion.

Neuronal (Ach), paracrine (histamine), and endocrine (gastrin) factors all regulate acid secretion. Their specific receptors (M_3, H_2 and CCK_2 receptors, respectively) are on the basolateral membrane of parietal cells in the body and fundus of the stomach. The histamine (H_2) receptor is a G-protein coupled receptor (GPCR) that activates the Gs-adenylcyclase-cyclic AMP-PKA pathway. Ach and gastrin signal through GPCRs that couple to the Gq-PLC-IP3-Ca2+ pathway in parietal cells. In parietal cells, the cyclic AMP and the Ca^{2+} - dependent pathways activate H^+,K^+ - ATPase (the proton pump), which exchanges hydrogen and potassium ions across the parietal cell membrane. This pump generates the largest known ion gradient in vertebrates, with an intracellular pH of about 7.3 and intracanalicular pH of about 0.8. (Bertram G. Katzung; 2006)

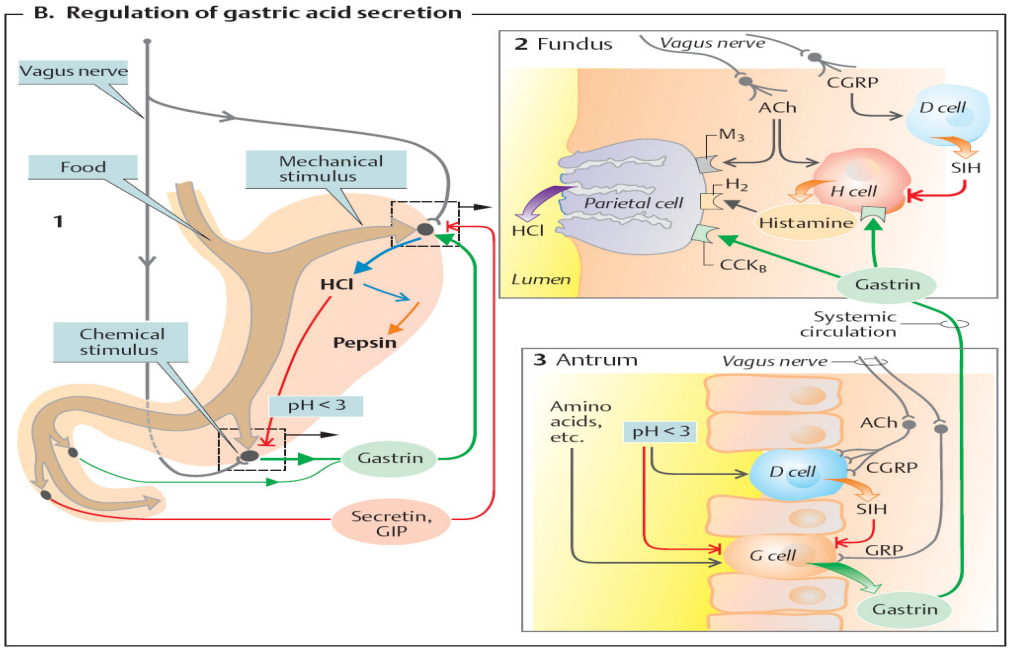


Fig 1.1: Regulation and Secretion of HCl in StomacH

Regulation of Secretion

Stomach acid secretion happens in several steps. Chloride and hydrogen ions are secreted separately from the cytoplasm of parietal cells and get combined into HCl only in their canaliculi. Gastric acid is then secreted into the lumen of the oxyntic gland and gradually reaches the main stomach lumen.

The highest concentration that it reaches in the stomach is 160 mM in the canaliculi. This is about 3 million times that of arterial blood, but almost exactly isotonic with other bodily fluids. The lowest pH of the secreted acid is about 0.8, but the acid gets diluted in the stomach lumen to the pH between 1 and 2.

At first, negative chloride ions and sodium ions get secreted actively from the cytoplasm of the parietal cell into the lumen of the canaliculus. This creates a negative potential of -40 mV to -70 mV across the membrane that enables the

diffusion of potassium ions and a small number of sodium ions from the cytoplasm into the canaliculus.

Another step is the production of hydrogen ions in the cytoplasm of parietal cells. The enzyme carbonic anhydrase catalyses the reaction between carbon dioxide and water, in which carbonic acid is produced. This acid immediately dissociates into hydrogen ions and hydrogen carbonate ions. The hydrogen ions leave the cell by the aid of H+/K+ ATPase antiporter.

At the same time sodium ions are actively reabsorbed. This means the largest amount of secreted K+ and Na+ ions return into the cytoplasm. In the canaliculus, secreted hydrogen and chloride ions combine into HCl and are then secreted into the lumen of the oxyntic gland.

There are three phases in the secretion of gastric acid:

1. **The cephalic phase**: 30% of the total gastric acid to be produced is stimulated by anticipation of eating and the smell or taste of food.

2. **The gastric phase**: 60% of the acid secreted is stimulated by the distention of the stomach with food. Plus, digestion produces proteins, which causes even more gastrin production.

3. **The intestinal phase**: the remaining 10% of acid is secreted when chyme enters the small intestine, and is stimulated by small intestine distention. (Ross & Wilson., 2006)

PEPTIC ULCER

Peptic ulcer disease (PUD) is characterized by inflamed lesions or excavations of the mucosa and underlying tissue of the upper gastrointestinal tract. The ulcers are the result of damage to the mucus membrane that normally protects the oesophagus, stomach and duodenum from gastric acid and pepsin. The pathophysiology of this gastro-intestinal disorder is viewed as an imbalance between mucosal defensive factors such as bicarbonate, prostaglandin, nitric oxide, peptides, growth factors and injurious factors like acid, pepsin. Gastric ulcer is often a chronic disease and may persist for 10 – 12 years characterized by repeated episode of healing and re-exacerbations**.(Coles, G.C., 1997.)**

Signs and symptoms

Symptoms of a peptic ulcer can be abdominal pain, classically epigastric with severity relating to mealtimes, after around 3 hours of taking a meal (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it) bloating and abdominal fullness; water brash (rush of saliva after an episode of regurgitation to dilute the acid in esophagus); nausea, and lots of vomiting; loss of appetite and weight loss; hematemesis (vomiting of blood); this can occur due to bleeding directly from a gastric ulcer, or from damage to the esophagus from severe/continuing vomiting melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin. Rarely, an ulcer can lead to a gastric or duodenal perforation. This is extremely painful and requires immediate surgery.

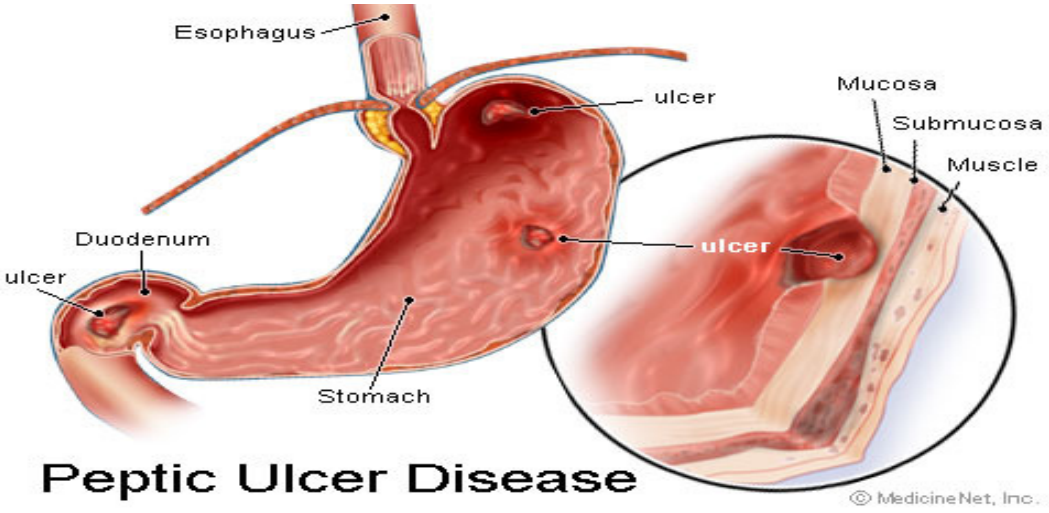


Fig 1.2: Types of Ulcers

A history of heartburn, gastroesophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion for peptic ulcer. Medicines associated with peptic ulcer include NSAID (non-steroid anti-inflammatory drugs) that inhibit cyclooxygenase, and most glucocorticoids (e.g. Dexamethasone and Prednisolone). In patients over 45 with more than two weeks of the above symptoms, the odds for peptic ulceration are high enough to warrant rapid investigation by EGD The timing of the symptoms in relation to the meal may differentiate between gastric and duodenal ulcers. A gastric ulcer would give

epigastric pain during the meal, as gastric acid is secreted, or after the meal, as the alkaline duodenal contents reflux into the stomach. Symptoms of duodenal ulcers would manifest mostly before the meal—when acid (production stimulated by hunger) is passed into the duodenum. However, this is not a reliable sign in clinical practice.

Complications

Gastrointestinal bleeding is the most common complication. Sudden large bleeding can be life-threatening. It occurs when the ulcer erodes one of the blood vessels. Perforation (a hole in the wall) often leads to catastrophic consequences. Erosion of the gastro-intestinal wall by the ulcer leads to spillage of stomach or intestinal content into the abdominal cavity. Perforation at the anterior surface of the stomach leads to acute peritonitis, initially chemical and later bacterial peritonitis. The first sign is often sudden intense abdominal pain. Posterior wall perforation leads to pancreatitis; pain in this situation often radiates to the back. Penetration is when the ulcer continues into adjacent organs such as the liver and pancreas.

Pathophysiology

The pathogenesis of duodenal ulcers (DU) and gastric ulcers (GU) is multifactorial and most likely reflects a combination of pathophysiologic abnormalities and environmental and genetic factors. Most peptic ulcers occur in the presence of acid and pepsin when Helicobacter pylori (HP), NSAIDs, or other factors disrupt normal mucosal defence and healing mechanisms. Acid is an independent factor that contributes to disruption of mucosal integrity. Increased acid secretion has been observed in patients with DU and may result from HP infection. Patients with GU usually have normal or reduced rates of acid secretion.

Alterations in mucosal defence induced by HP or NSAIDs are the most important cofactors in peptic ulcer formation. Mucosal defence mechanisms include mucus and bicarbonate secretion, intrinsic epithelial cell defence, and mucosal blood flow. Maintenance of mucosal integrity and repair is mediated by endogenous

prostaglandin production. H. pylori infection causes gastritis in all infected individuals and is causally linked to PUD. Most non-NSAID ulcers are infected with HP, and HP eradication markedly decreases ulcer recurrence. H. pylori may cause ulcers by direct mucosal damage, impairing mucosal defence via elaboration of toxins and enzymes, altering the immune/inflammatory response, and by increasing antral gastrin release, which leads to increased acid secretion.

Nonselective NSAIDs (including aspirin) cause gastric mucosal damage by two mechanisms: (1) a direct or topical irritation of the gastric epithelium, and (2) systemic inhibition of the cyclooxygenase-1 (COX-1) enzyme, which results in decreased synthesis of protective prostaglandins. Use of corticosteroids alone does not increase the risk of ulcer or complications, but ulcer risk is doubled in corticosteroid users taking NSAIDs concurrently. Epidemiologic evidence links cigarette smoking to PUD, impaired ulcer healing, and ulcer-related GI complications. The risk is proportional to the amount smoked per day.

Although clinical observation suggests that ulcer patients are adversely affected by stressful life events, controlled studies have failed to document a cause-and-effect relationship. Coffee, tea, cola beverages, beer, milk, and spices may cause dyspepsia but do not increase PUD risk. Ethanol ingestion in high concentrations is associated with acute gastric mucosal damage and upper GI bleeding but is not clearly the cause of ulcers.

Macroscopical appearance

Gastric ulcers are most often localized on the lesser curvature of the stomach. The ulcer is a round to oval parietal defect ("hole"), 2 to 4 cm diameter,

with a smooth base and perpendicular borders. These borders are not elevated or irregular in the acute form of peptic ulcer, regular but with elevated borders and inflammatory surrounding in the chronic form. In the ulcerative form of gastric cancer the borders are irregular. Surrounding mucosa may present radial folds, as a consequence of the parietal scarring.

Microscopical appearance

A gastric peptic ulcer is a mucosal defect which penetrates the muscularis mucosae and muscularis propria, produced by acid-pepsin aggression. Ulcer margins are perpendicular and present chronic gastritis. During the active phase, the base of the ulcer shows 4 zones: inflammatory exudate, fibrinoid necrosis, granulation tissue and fibrous tissue. The fibrous base of the ulcer may contain vessels with thickened wall or with thrombosis.

Epidemiology

1. Peptic ulcer is the most common, chronic gastrointestinal disorder and has become a common global health problem affecting a large number of people worldwide and still a major cause of morbidity and mortality **(Alder R.,1984)** Geographically, the disease is prevalent throughout the work, in USA annually 3.7 million people are affected by this disease **(Beckette, A.H. and Stenlake**, **J.B.,1997).**

Types of Ulcer

- **Gastric ulcer**

When a peptic ulcer is in the stomach, it is called as gastric ulcer. The symptoms of gastric ulcers are more specific than peptic ulcer symptoms.

- **Duodenal ulcer**

When a peptic ulcer is in the duodenum, it is called a duodenal ulcer. This type of peptic ulcer develops in the first part of the small intestine. Some of the symptoms of a duodenal ulcer are interestingly quite opposite to those of gastric ulcer.

- **Oesophageal ulcer**

This type of ulcer occurs in the lower end of the oesophagus. Oesophageal ulcers are often associated with a bed case of acid reflux or GERD as it is commonly called.

- **Bleeding ulcer**

Internal bleeding is caused by a peptic ulcer which has been left untreated. When this happens, it is now referred to as bleeding ulcer – this is the most dangerous type of ulcer.

Refractory ulcer

Refractory ulcers are simply peptic ulcers that have not healed after atleast 3 months of treatment.

Stress ulcer

Stress ulcers are a group of lesion (or laceration) found in the oesophagus, stomach or duodenum. These are normally only found in critically ill or severely stressed patients.

Factors causing peptic ulceration

Gastric injury induced by NSAIDs

Non steroidal anti-inflammatory drugs (NSAIDs) are used widely in the treatment of pain, fever, inflammation, rheumatic and cardiovascular disease and more recently for the prevention of colon cancer and Alzheimer's disease. Chronic administration of these drugs is often associated with the development of adverse gastrointestinal effects, such as gastric erosions, gastric and duodenal ulceration and severe complications such as gastrointestinal hemorrhage and perforation. NSAIDs like aspirin, indomethacin is known to induce ulcer by inhibition of cyclooxygenase enzyme (COX) and suppression of prostagladin (PG) mediated effects on mucosal protection. Endogenous prostaglandins regulate the mucosal blood flow, epithelial cell proliferation, epithelial reconstitution, mucosal immunocyte function, mucous and bicarbonate secretion. Beside, these neutrophil and oxygen radical – dependant microvascular injuries may be important processes that lead to mucosal damage in response to NSAIDs administration.

These agents cause; the activation of neutrophils and their adherence to the vascular endothelium, hence blocking capillaries and reducing local gastric blood flow. It also reduces the hydrophobicity of the mucus gel layer by changing the

action of surface – active phospholipids. These drugs cause an increase in acid and pepsinogen secretion**.(Plant Drug Evaluation, Divakar M.C., 1996,).**

Stress

Stress ulcers are single or multiple mucosal defects which can become complicated by upper gastrointestinal bleeding during the physiologic stress of serious illness. Ordinary peptic ulcers are found commonly in the gastric antrum and the duodenum whereas stress ulcers are found commonly in fundic mucosa and can be located anywhere within the stomach and proximal duodenum.

Stress plays an important role in the etiopathology of gastro-duodenal ulceration. Increase in gastric motility, vagal over activity; mast cell degranulation; decreased gastric mucosal blood flow; and decreased prostaglandin synthesis

Gastric stress ulceration is probably mediated by release of histamine with enhanced secretion of acid and reduced mucus production.

Helicobacter pylori

Helicobacter pylori are a gram-negative, microaerophilic bacterium that inhabits various areas of the stomach and duodenum. It causes a chronic low-level inflammation of the stomach lining and is strongly linked to the development of duodenal and gastric ulcers and stomach cancer. Over 80% of individuals infected with the bacterium are asymptomatic. Gastroduodenal diseases caused by H.pylori are associated with infiltration of gastric mucosa by neutrophil, lymphocyte, monocytes and plasma cell. **(Coles, G.C., 1997).**

Smoking

Smoking and nicotine have significant adverse effects. Smoking and chronic nicotine treatment stimulates basal acid output which is more pronounced in the smokers having duodenal ulcer. This increased gastric acid secretion is mediated through the stimulation of H_2-receptor by histamine released after mast cell degranulation and due to the increase of the functional parietal cell volume or secretory capacity in smokers. Smoking and nicotine stimulate pepsinogen secretion also by increasing chief cell number or with an enhancement of their secretory capacity. Long-term nicotine treatment in rats also significantly decreases total mucus neck cell population and neck-cell mucus volume.

Smoking also increases bile salt reflux rate and gastric bile salt concentration thereby increasing duodeno-gastric reflux that raises the risk of gastric ulcer in smokers. Smoking and nicotine not only induce ulceration, but they also potentiate ulceration caused by H. pylori, alcohol, nonsteroidal anti-inflammatory drugs or cold restrain stress. Polymorphonuclear neutrophils (PMN) play an important role in ulcerogenesis through oxidative damage of the mucosa by increasing the generation of reactive oxygen intermediates (ROI), which is potentiated by nicotine and smoking.

Smoking increases production of platelet activating factor (PAF) and endothelin, which are potent gastric ulcerogens. Nicotine also decreases prostaglandin generation in the gastric mucosa of smokers, thereby making the mucosa susceptible to ulceration. ROI generation and ROI-mediated gastric mucosal cell apoptosis are also considered to be important mechanism for aggravation of ulcer by cigarette smoke or nicotine.

Both smoking and nicotine reduce angiogenesis in the gastric mucosa through inhibition of nitric oxide synthesis thereby arresting cell renewal process. Smoking or smoke extract impairs both spontaneous and drug-induced healing of ulcer. Smoke extract also inhibits gastric mucosal cell proliferation by reducing ornithine decarboxylase activity, which synthesizes growth-promoting polyamines. It is concluded that gastric mucosal integrity is maintained by interplay of some aggressive and defensive factors controlling apoptotic cell death and cell proliferation and smoking potentiates ulcer by disturbing this balance.(**Hiruma-lima *et.,al.1994)***

Blood group

There is increasing evidence that blood group substances play a role in the causation of disease or in the protective mechanism against it. The incidence of peptic ulcer is higher in the people having blood group "O" than those of blood groups A and B **(Coles, G 1997.,)**

Sex

Duodenal ulcer is seen more commonly in man than women, though gastric ulcer occurs to an equal extent in man and women.(**Bowman** *et al.,*1980)

Dietary factors

Caffeine

Caffeine can stimulate acid secretion in stomach and can aggravate an existing ulcer.

Alcohol

There is little evidence of an association between alcohol use and peptic ulcer. Ulcers are common in people who have cirrhosis of the liver, a disease often linked to heavy alcohol consumption.

Dietary salt

The evidence was found between an association of gastric ulcer and dietary salts. The occurrence of gastric ulcer may be linked to the amount of dietary salt consumption. The salt was shown to induce gastritis of experimental animals. (**Coles G1997**.)

Treatment

Non Pharmacologic Treatment

- Patients with PUD should eliminate or reduce psychological stress, cigarette smoking, and the use of NSAIDs (including aspirin). If possible, alternative agents such as acetaminophen, a nonacetylated salicylate (e.g., salsalate), or COX-2 selective inhibitor should be used for pain relief.
- Although there is no need for a special diet, patients should avoid foods and beverages that cause dyspepsia or exacerbate ulcer symptoms (e.g., spicy foods, caffeine and alcohol).

Pharmacologic Treatment

- Eradication of HP is recommended for HP-infected patients with GU, DU, ulcer-related complications, and in some other situations. Treatment should be effective, well tolerated, easy to comply with, and cost-effective.

- First-line eradication therapy is a proton pump inhibitor (PPI)-based three-drug regimen containing two antibiotics. Most clinicians prefer clarithromycin and amoxicillin, reserving metronidazole for back-up therapy. The PPI should be taken 15 to 30 minutes before meals along with the two antibiotics. Although an initial 7-day course provides minimally acceptable eradication rates, longer treatment periods (10 to 14 days) are associated with higher eradication rates and less antimicrobial resistance.

- First-line treatment with quadruple therapy using a PPI (with bismuth, metronidazole, and tetracycline) achieves similar eradication rates as PPI-based triple therapy and permits shorter treatment duration (7 days). However, this regimen is often recommended as second-line treatment when a clarithromycin-amoxicillin regimen is used initially. All medications except the PPI should be taken with meals and at bedtime.

- If the initial treatment fails to eradicate HP, second-line empiric treatment should: (1) use antibiotics that were not included in the initial regimen; (2) include antibiotics that do not have resistance problems; (3) use a drug that has a topical effect (e.g., bismuth); (4) be extended to 10 to 14 days. Thus, if a PPI-clarithromycin-amoxicillin regimen fails, therapy should be instituted with a PPI, bismuth subsalicylate, metronidazole, and tetracycline for 10 to 14 days.

- Conventional treatment with standard dosages of H_2-receptor antagonists (H_2RA) or sucralfate alone (without antibiotics) relieves ulcer symptoms and heals most gastric and duodenal ulcers in 6 to 8 weeks. PPIs provide comparable healing rates over 4 weeks. However, when conventional antiulcer therapy is discontinued after ulcer healing, most HP-positive patients develop a recurrent ulcer within 1 year.

- Maintenance therapy with a PPI, low-dose H_2RA, or sucralfate may be indicated for patients who have a history of ulcer-related complications, a healed refractory ulcer, failed HP eradication therapy, or who are heavy smokers or NSAID users.

- Most uncomplicated NSAID-induced ulcers heal with standard regimens of an H_2RA, PPI, or sucralfate if the NSAID is discontinued. If the NSAID must be continued, consideration should be given to reducing the NSAID dose or switching to acetaminophen, a nonacetylated salicylate, a partially selective COX-2 inhibitor, or a selective COX-2 inhibitor. PPIs are the drugs of choice when NSAIDs must be continued because potent acid suppression is required to accelerate ulcer healing. If HP is present, treatment should be initiated with an eradication regimen that contains a PPI. Patients at risk of developing serious ulcer-related complications while on NSAIDs should receive prophylactic cotherapy with misoprostol or a PPI.

- Patients with ulcers refractory to treatment should undergo upper endoscopy to confirm a nonhealing ulcer, exclude malignancy, and assess HP status. HP-positive patients should receive eradication therapy.

LITERATURE REVIEW

- **P. Thirunavukkarasu** ***et.al.,(1987)*** studied The plant extract of Excoecaria agallocha bark herbal preparation that has been suggested as usefulin the treatment of varies diseases (anti tumor, anti microbial, anti wound killing agents and anti oxidant). In this, study to determine the gastro protective effect of E. agallocha in a model of NSAID induced ulcer rat. Thelyophilized extract was given by oral gavages (125 and 62.5mg/kg) three times at 12 h intervals before administering dicolofenac 100mg/kg. Pretreatment with the extract resulted in a significant decreased of theulcerated area. The volume and acidity of the gastric juice decreased in the pretreated rats. The plantextract was elevated in the gastric juice of untreated rats, showed hear normal levels in the pretreated rats.The E. agallocha was able to decrease the acidity and increase the mucosal defense in the gastric areas, thereby justifying its use as an antiulcerogenic agent.

- **T Lakshmi Srinivas**, ***et.al., (2001)*** studied Peptic ulcer disease (PUD) is considered as one of the common diseases in the world. Treatment of peptic ulcer with synthetic drugs such as proton pump inhibitors, H2 receptor antagonists and other non-steroidal anti-inflammatory drugs has shown adverse effects, relapses, drug interactions. Medicinal plants containing active chemical constituents are useful in prevention and treatment of various diseases. Literatures suggest that polyherbal formulations of medicinal plants are considered to be potential source for the treatment of ulcers. Combination of ayurvedic knowledge with modern medicine can produce better antiulcer drugs of natural origin from medicinal plants with fewer side effects. This study has presented the review of commonly used anti-ulcer plants which are used for the

treatment or prevention of peptic ulcers and the other reported activities of these plants.

- **Nusrat subhani *et.al., (1984)*** studied The effect of alcoholic extracts of bark from Excoecaria. agallocha Linn. (Family: Euphorbiaceae) was evaluated in experimental models of pain and ulceration. Crude extracts of Excoecaria agallocha (300 mg/kg dose) showed maximum time needed for the response against thermal stimuli (6.72±0.43 seconds) which is comparable to diclofenac sodium (8.20±0.21seconds) in the hot plate test. Hot tail immersion test also showed similar results as in hot plate test. The bark extracts at 500 and 250 mg/kg showed significant reduction in acetic acid induced writhings in mice with a maximum effect of 53.87% reduction at 500 mg/kg dose. The effect produced by the alcoholic extract at the highest dose was comparable to that of diclofenac sodium at 100 mg/kg (70.56%). It has also been seen anti-ulcerogenic activity compared to acetylsalicylic acid, which may be due to the protective effect of the extract. The result suggest that the analgesic effect of the extract as claimed in folklore medicine, which may be mediated via both peripheral and central mechanism having gastro-protective effect.

- **SVarahalarao Vadlapudi1 *et.al. (1999),*** studied Excoecaria agallocha L. leaves were extracted by various extracting procedures, using different solventsfor testing the antimicrobial activities against important microorganisms using agar well diffusion method. Chloroform and methanolic extracts were found to be effective against these organisms, whereas hexane extracts were inactive. The purpose of this study was to find preliminary data for the development of

alternative treatments to chemical microbicides for the control of plant diseases from natural plant extracts

- **Muhammed Ashraf *et.al. (2007),*** studied The objective of this study was to investigate the gastro protective activity of ethanolic extract of leaves of Ficus pumila L. (Moraceae) in different experimental models of gastric ulcer in rats. Two doses (200 mg/kg and 400 mg/kg) of the extracts were used for the study. The experimental ulcers were induced by different models such as pyloric ligation, ethanol and cold restrained ulcer models. Omeprazole (10 mg/kg) and sucralfate 100 mg/kg) were used as the standard drugs. All drugs were administered by the oral route. The ulcer index and percentage of ulcer protection were measured. Phytochemical tests and various parameters such as free acidity, total acidity, total hexose, Hexosamine, Fucose and total protein were measured.

- **Pal S *et.al., (1997)*** studied A methanolic fraction from an extract of Bryophyllum pinnatum leaves was found to possess significant anti-ulcer activity in nine different experimental animals models. Premedication tests in rats revealed that the extract possessed significant protective action against the gastric lesions induced by aspirin, indomethacin, serotonin, reserpine, stress and ethanol. Significant protection with extract treatment was observed to occur for aspirin-induced ulcer in pylorus-ligated rats and for histamine-induced duodenal lesions in guinea pigs. Significant enhancement of the healing process was also found to occur in acetic acid-induced chronic gastric lesions in rats.

- **Paul V Tan *et al*., (2001)** analyzed the anti-ulcerogenic effects of the leaf methanol extract of *Ocimum suave using* four ulcer models in wistar rats:

HCl/ethanol-induced ulcer, Absolute ethanol induced ulcer, indomethacin-HCl/ethanol-induced ulcer, pylorus ligation ulcers. Administration of the extract of O.*suave* to the rats by oral route (75-500mg/kg) dose dependently reduced the formation of gastric ulcers in all four models, accompanied by significant increase in gastric mucus production. At the dose of 250mg/kg there was complete inhibition of gastric lesions induced by HCl/ethanol ulcers. Pre-treatment, by indomethacin i.p. significantly reduced the ability of the extract to inhibit the formation of HCl/ethanol lesions. In PL model, the dose of 500mg/kg completely inhibited lesion formation but did not have effect on gastric secretion compared with the controls. The mucus secretion promoting effect of the extract was most significant when gastric environment was highly acidic.

- **Jeetendra Kumar Gupta *et al.*, (2002)** studied Anti ulcer activity of *Leucas lavandulifolia* on mucosal lesion in rats. The reduction of ulcer index as well as gastric acid output in extract treated animals was found to be statistically significant with respect to control animals. The extract exhibited ulcer protection activity in dose dependent manner.

- **C. Penido *et al., (2001)*** studied the anti-inflammatory and gastroprotective properties of ethanolic extracts of *Stachytarpheta cayennesis* (L.C. Rich) Vahl (Verbenaceae). Chromatographic analysis of the crude ethanolic extract, SC01, revealed high concentrations of the iridoid ipolamide, whereas the SC02, the second ethanolic extract, presented the arylpropanoid verbacoside as a major constituent. The extract SC02 presents an important anti-ulcerogenic activity, since it inhibited diclofenac-induced (100 mg/kg, p.o.) gastric ulcer.

- **Saroj K. Pal *et. al., (1994)*** studied The methanol fraction of *M. oleifera* leaf extract was found to possess significant protective actions in acetylsalicylic acid, serotonin and indomethacin induced gastric lesions in experimental rats. A significant enhancement of the healing process in acetic acid—induced chronic gastric lesions was also observed with the extract-treated animals.

- **G.Vinothapooshan *et. al (1994)*** studies The effect of Methanolic, chloroform and diethel ether extracts of Mimosa pudica was investigated in rats to evaluate the anti-ulcer activity by using three models, i.e. Aspirin, Alcohol and pyloric ligation models experimentally induced gastric ulcer. The parameters taken to assess anti-ulcer activity were volume of gastric secretion, PH, free acidity, total acidity and ulcer index. The results indicate that the alcoholic extract significantly ($P < 0.001$) decreases the volume of gastric acid secretion, PH, free acidity, total acidity and ulcer index with respect to control

- **Dr. Prasanta Kumar Mitra *et.al(2002)*** studied An active compound (SP-1) was isolated from the seeds of Nirmali (Strychnor potatorum Linn.) and its antiulcer activity was studied against ethanol, hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats. A significant antiulcer activity of SP-1was observed in all the models. SP-1 thus provides a scientific rationale for the use as antiulcer drug

- **Marslin Gregory *et. al (1989)*** studied : To evaluate the anti-ulcer activity and acute toxicity of Ficus religiosa (F. religiosa) leaf ethanolic extract in animal models. Methods: Anti-ulcer activity of F. religiosa ethanolic extract (250 and 500 mg/kg body weight) was studied on stress induced ulcer animal models. Ranitidine was used as standard. The anti-ulcer activity of F. religiosa was

evaluated with the help of ulcer area and histopatholgical examination. Preliminary phyto-chemical screening and acute toxicity studies of F. religiosa also carried out

- **M.TUORKEY *et.al (2001)*** studied The rats were divided into four groups and fasten for 2 days with free access to water. On the third day, the animals were fasted for a further 24 h with no access to water followed by surgery. Rats received different doses of curcumn (20, 40, and 80 mg/kg) or vehicle by oral gavage. Nineteen hours after ulcer induction, the rats were killed by decapitation. Stomach was opened along the greater curvature and ulcerative lesions were counted. Total juice acidity, neutrophils activity, mitochondrial activity, total antioxidants, paraoxonase (PON 1)/arylesterase and total peroxides were evaluated. DNA fragmentation (%) and pro-inflammatory cytokine IL6 level were measured. The level of different gastrocytoprotective effectors including total antioxidants and paraoxonase (PON 1)/arylesterase activities was measured

- **Hitesh Kumar Kinger *et.al (2007)*** studied Polygonum barbatum (Polygonaceae) is a plant, reported for its variety of ethnic medicinal uses. Hence we have planned to screen antiulcer activity of whole plant with the alcoholic and aqueous extracts. Whole plant was successively extracted with alcohol and water was subjected for phytochemical screening to identify different phytoconstituents. Ld50 studies for both (alcoholic and aqueous) extracts were conducted upto the dose level of 2 g /kg by following OECD up and down method of guidelines No.425. Anti-ulcer activity was evaluated in various animal models like Pylorus ligation, Ethanol Induced gastric mucosal

damage ulcer modelsin rats. Preliminary phytochemical studies revealed the presence of saponins, sterols, mucilage, glycosides, alkaloids, steroidal saponins in both the alcoholic and aqueous extracts of P. barbatum. No mortality was observed with any of the 2 extracts up to the maximum dose level of 2 g/kg. Further alcoholic and aqueous extracts at 200 and 400 mg/kg, p.o but not with 100 mg/kg p.o doses significantly ($P < 0.01$) reduced the ulcer score, ulcer number, ulcer index, free acidity and total acidity in Pylorus ligation, Ethanol Induced gastric mucosal damage ulcer models in rats.The present study revealed the antiulcer activity of whole plant extracts of P. barbatum and the activities are due to the presence of phytochemical constituents such as saponins, sterols, mucilage, glycoside, alkaloids, steroidal saponins as these phytochemical constituents were already reported for the above mentioned effects.

AIM AND PLAN OF WORK

The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs.

In India, PUD is common. In the Indian Pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share. Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and second with reinforcing gastric mucosal barrier.(**Dharmani p *et al.,2006)***

A number of drugs including proton pump inhibitors and H_2 receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects like arrhythmias, impotence, gynaecomastia, arthralgia, hypergastrinemia and haemopoeitic changes and drug interactions. This has been the rationale for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse.

PLAN OF WORK

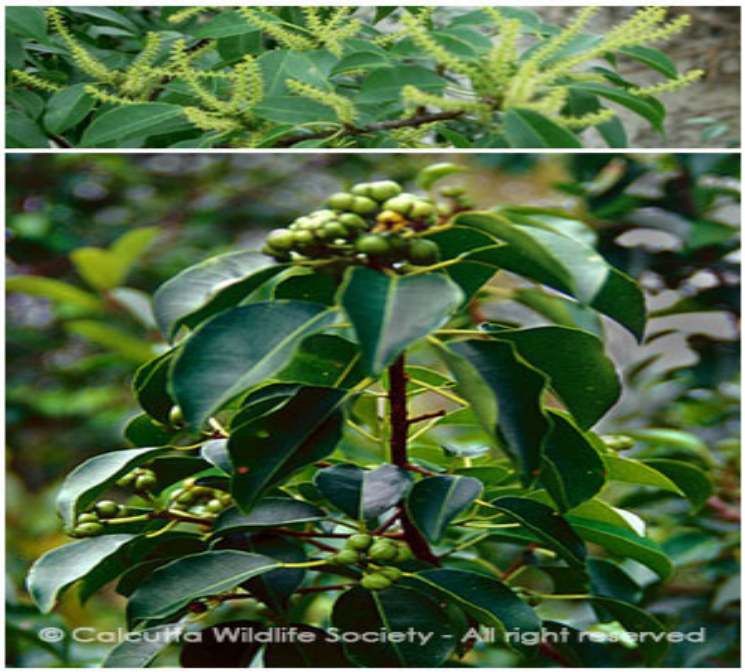
The plan of work for the study of ***Excoecaria agallocha linn*** was carried out as follows.

1. **Collection and authentification of plant.**
2. **Pharmacognostical studies**
    a. Ash value
    b. Extractive value
    c. Fluorescence analysis
    d. Foaming index
3. **Preliminary phytochemical studies**
    a. Preparation of extract
    b. Qualitative phytochemical studies

PHARMACOLOGICAL STUDIES

1. **Acute Oral Toxicity studies**
2. **Evaluation of anti-ulcer activity of *Excoecaria agallocha* by**
    - Ethanol induced ulcer model
    - Stress induced ulcer model
3. **Determination of following parameters**
    - Ulcer index
    - Percentage inhibition
    - Gastric volume
    - pH of gastric juice
    - Total acidity
    - Free acidity

PLANT PROFILE



Fir 4.1: Plant image of *Excoecaria agallocha linn*

BOTANICAL INFORMATION:

Excoecaria agallocha linn

Family : Euphorbiaceae

Synonyms : commia glyphostylus

Common names

Thillai, milky mangrove, blind-your-eye mangrove and river poison tree.

Taxonomical classification

Kingdom : Plantae-Plants

Subkingdom : Tracheobionta

Division : Magnoliopsida

Class : Malpighiales

Family : Euphorbiaceae

Genus : Excoecaria

Species : Excoecaria agallocha linn

Description

Thillai is a tree, usually not more than 8 meters high. Leaves are alternate, shiny, pointed at the top, somewhat rounded at the base, elliptic-ovate, oblong-ovate or ovate, and 6 to 12 centimeters long. Flowers are very small, densely crowded on slender and flowering branches. Male flowers occur on spikes which grow singly in the axils of leaves, from 5 to 10 centimeters long. Female flowers are borne branches, 2 to 3 cm long. Sepals are three with a basal gland within, no petals, three

stamens. Fruits is somewhat rounded, smooth, about 5 millimeters in diameter, three sections. Along the seashore or any place reached by salt or brackish water throughout the Philippines.Also occurs in India to Polynesia

Properties:

Milky juice is caustic and poisonous; causing temporary blindness to the eye and blistering of the skin.

Parts used

Bark, leaves, flowers and roots are used.

Constituents

Six triterpenoids including taraxerone (1), beta-amyrin acetate (2), 3beta-[(2E,4E)-6-oxo-decadienoyloxy]-olean-12-ene (3), taraxerol (4), acetylaleuritolic acid (5), and cycloart-22-ene-3beta, 25-diol (6), and three steroids including beta-sitostenone, (24R)-24-ethylcholesta-4,22-dien-3-one, and beta-sitosterol.

Medicinal use

Latex used in healing of obstinate ulcers. Decoction of leaves used for epilepsy also applied to ulcers.Roots less poisonus than above-ground parts,are pounded with ginger and used to make embrocating forswellings of the hands and feet.Bark and wood used for flatulence.In india,seed poultice used for crippling arthritis.

MATERIALS AND METHODS

Collection of Plant

Plant was collected from thillai, chidambaram, Tamilnadu in the month of march-july . The fresh leaves of the plant were separated from adulterants, shade dried, broken into small pieces and powdered coarsely.

PHARMACOGNOSTIC STUDIES

ASH VALUE (Kokate *et al*,1985)

Principle

The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. There is a considerable difference varies within narrow limits in the case of the same individual drug. Hence an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. Ash standards have been established for a number of official drugs. Usually these standards get a maximum limit on the total ash or on acid insoluble ash permitted.

The total ash is the residue remaining after incineration. The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid.

The ash or residue yielded by an organic chemical compound is as a rule, a measure of the amount of inorganic matters present as impurity. In most cases, the inorganic matter is present in small amounts which are difficult to remove in the

purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drugs in powder form.

Procedures given in Indian pharmacopoeia were used to determine the different ash values such as total ash and acid insoluble ash.

Total ash

Weighed accurately about 3 gm of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red until free from carbon, cooled and weighed and then calculated the percentage of total ash with reference to the air dried drug.

Acid insoluble ash

The ash obtained as directed under total ash above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on ashless filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Water soluble ash

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450˚C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash is calculated with reference to the air dried drug.

EXTRACTIVE VALUES

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of alcohol soluble extractive value

5 gm of the air-dried coarse powder of ***Excoecaria agallocha linn*** was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18hours. Thereafter, it was filtered rapidly taking precautions against the loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air- dried drug.

Determination of water soluble extractive value

Weigh accurately 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow to standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

LOSS ON DRYING

Loss on drying is the loss in weight in percentage w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Desiccators or hot air oven). If the sample is in the form of large crystals, then reduce the size by quick crushing to a powder.

Procedure

About 1.5 gm of powdered drug was weighed accurately in a tarred porcelain dish which was previously dried at 105˚C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated. The results are recorded in the table.

FOAMING INDEX:

Foaming index is mainly performed to determine the saponin content in an aqueous decoction of plant material.

Determination of foaming index:

Weighed accurately about 1g of coarsely powdered drug and transformed to 500ml conical flask containing 100ml of boiling water. Maintained at moderate boiling at 80-90˚c for about 30min. Cooled and added sufficient water through the filter to make up the volume to 100ml (V1). Cleaned 10 stoppered test tube of uniform dimension were taken and transferred the successive portions of 1,2,3ml upto 10ml and adjusted the volume of the liquid in each test tube with water to 10ml.Stoppered the tubes and shaken them in a lengthwise motion for 15 sec

uniformly and allowed to stand for 15min and measure the height of foam. If the height of the foam in every tube is less than 1cm, the foaming index is less than 100(not significant). Here the foam was more than 1cm height after dilution of plant material. If the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of first decoction of plant material is measured and transferred to 100ml volumetric flask (V2) and volume is made to 100ml and followed the same procedure.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Extraction of plant material

- **Ethanolic extraction**

About 400g of air dried powdered material was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days. At the end of second day the powder was taken out and it was dried. After drying it was again packed and extracted by using ethanol as solvent, till colour disappeared. The temperature was maintained at 55ºC-65ºC. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield of ethanolic extract were noted.

Table 5.1:Nature and colour of extract Excoecaria agallocha linn

S.No.	*Name of extract*	*Colour*	*Consistency*	*Yield% w/w*
1	*ethanolic extract*	*Dark brown*	*Sticky mass*	*11.67*

CHEMICAL TESTS

A) Test for carbohydrates

1**. Molisch Test:** It consists of treating the compounds of a-naphthol and concentrated sulphuric acid along the sides of the test tube.

Purple colour or reddish violet colour was produced at the junction between two liquids. **(Kokate** *et.al.,* 2009)

2**. Fehling's Test:** Equal quantity of Fehling's solution A and B is added. Heat gently, brick red precipitate is obtained.

3**. Benedict's test:** To the 5ml of Benedict's reagent, add 8 drops of solution under examination. Mix well, boiling the mixture vigorously for two minutes and then cool. Red precipitate is obtained.

4. **Barfoed's test:** To the 5ml of the Barfoed's solution add 0.5ml of solution under examination, heat to boiling, formation of red precipitate of copper oxide is obtained.

B) Test for Alkaloids

1. **Dragendroff's Test:** To the extract, add 1ml of Dragendroff's reagent Orange red precipitate is produced.

2. **Wagner's test:** To the extract add Wagner reagent. Reddish brown precipitate is produced.

3. **Mayer's Test:** To the extract add 1ml or 2ml of Mayer's reagent. Dull white precipitate is produced.

4. **Hager's Test:** To the extract add 3ml of Hager's reagent yellow precipitate is produced.

C) Test for Steroids and Sterols

1. **Liebermann Burchard test:** Dissolve the test sample in 2ml of chloroform in a dry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

2. **Salkowski test:** Dissolve the sample of test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish red, cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

D) Test for Glycosides

1. **Legal's test:** Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red colour is produced.

2. **Baljet test:** To the drug sample, sodium picrate solution is added. Yellow to orange colour is produced.

3. **Borntrager test:** Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.

4. **Killer Killani test:** Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

E) Test for Saponins

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes.1 cm layer of foam indicates the presence of saponins.

F) Test for Flavonoids

Shinoda test: To the sample, magnesium turnings and then concentrated hydrochloric acid is added. Red colour is produced.

G) Test for Tri-terpenoids

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

H) Tests for Tannins and Phenolic Compounds

The Phenol content in the raw material of extract was estimated spectroscopically. To 2-3 ml of extract, add few drops of following reagents:

a). 5% $FeCl_3$ solution: deep blue-black color.

b). Lead acetate solution: white precipitate.

c). Gelatin solution: white precipitate

d). Bromine water: decolouration of bromine water.

e). Acetic acid solution: red color solution

f). Dilute iodine solution: transient red color.

g). Dilute HNO_3: reddish to yellow color.

I) Test for Fixed Oils and Fatty acids

1. **Spot test:** Small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

2. **Saponification test:** Few drops of 0.5N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution. Later the mixture is heated on water bath for 1-2 hours soap formation indicates the presence of fixed oils and fats in the extracts.

J) Test for Gums and Mucilage

Ruthenium red test: Small quantities of extract are diluted with water and added with ruthenium red solution. A pink colour production shows the presence of gums and mucilage.

K) Test for Proteins and Amino acids

1. **Biuret test:** Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.
2. **Ninhydrin test:** Add 2 drops of freshly prepared 0.2% Ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.
3. **Xanthoprotein test:** To the extract, add 20% of sodium hydroxide or ammonia. Orange colour indicates presence of aromatic amino acid.

TOXICOLOGICAL EVALUATION

Determination of LD_{50} value of *Excoecaria agallocha linn*

Acute Oral Toxicity Study

The procedure was followed by using OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion. All the experimental procedures were reviewed and in accordance with the recommendations for the proper care and use of laboratory animals.

The method uses defined doses (5, 50, 300, 2000 mg / kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized system (GHS) for the classification of chemical which cause acute toxicity.

Procedure

Three animals (Albino mice, 25-75gm) were selected for studies.

The ethanolic extracts of ***Excoecaria agallocha linn***, was administered through oral route.

Most of the crude extracts possess LD_{50} value more than 2000 mg. /kg of the body weight of animal used.

Dose volume was administered 0.1 ml / 100 gm body weight to the animal by oral route. After giving the dose the toxic signs were observed within 3-4 hours.

Body weight of animals before and after administration, onset of toxicity and signs of toxicity like changes in skin and fur, eyes, and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavior pattern, signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma was also to be noted, if any, was observed.

PHARMACOLOGICAL EVALUATION

EVALUATION OF ANTI-ULCER ACTIVITY

Animals

Male Albino rats, weighing 150-200g were used in the present study. All the rats were kept at room temperature (22°C) in the animal house. All the animals were housed and treated as per the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food and were acclimatized to laboratory conditions. All the experimental procedures were reviewed and in accordance with the recommendations for the proper care and use of laboratory animals.

Experimental procedure

ETHANOL INDUCED ULCER

Male Albino rats were divided in to six groups of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy. Six groups were made as below.

Group I - received 1% Acacia (1.0ml/kg p.o) as normal control.

Group II - received 1% Acacia (1.0ml/kg p.o) as vehicle control.

Group III - received (100mg/kg, p.o) ethanol extract of ***Excoecaria agallocha linn***

Group IV - received (200mg/kg, p.o) ethanol extract of ***Excoecaria agallocha linn***

Group V - received (400mg/kg,p.o) ethanol extract of ***Excoecaria agallocha linn***

Group VI - received (20mg/kg, p.o) sucralfate as standard

One hour after the drug treatment the animals were treated with absolute ethanol [5ml/kg] to induce ulcers. The animals were sacrificed after 1hr and stomach was opened and percentage inhibition of ulcer was determined**.(Bertram G. Katzung; Basic and Clinical Pharmacology, 10th edition, 2006).**

SWIMMING STRESS INDUCED ULCER

Stress ulcers were induced by forced swimming in the glass cylinder (height 45cm, diameter 25cm) containing water to the height of 35cm maintained at 25ºC for 3 hours. Male Albino rats were divided into six groups of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy. Six groups were made as below

Group I - received 1% Acacia (1.0ml/kg p.o) as normal control.

Group II - received 1% Acacia (1.0ml/kg p.o) as vehicle control.

Group III - received (100mg/kg, p.o) ethanol extract of ***Excoecaria agallocha linn***

Group IV - received (200mg/kg, p.o) ethanol extract of ***Excoecaria agallocha linn***

Group V - received (400mg/kg, p.o) ethanol extract of ***Excoecaria agallocha linn***

Group VI - received (20mg/kg, p.o) Omeprazole as standard

After the drug treatment animals were allowed to swim in water for 3 hours. Then the animals were sacrificed and stomach was opened. The ulcer index and percentage inhibition of ulcer was determined.

BIOCHEMICAL PARAMETERS

The stomach was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged. The gastric contents were analyzed for gastric juice volume, pH, free and total acidity.

Measurement of gastric juice volume and pH

Gastric juice was collected from ethanol induced ulcer rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100g body weight. The pH of the supernatant was measured using digital pH meter. (Bertram G. Katzung; Basic and Clinical Pharmacology, 10^{th} edition, 2006).

Determination of free and total acidity

An aliquot of 1.0 ml of gastric juice was pipette out in to a 250 ml conical flask and 2/3 drops of Topfers reagent was added to it and titrated with 0.01N NaOH until all traces of the red colour disappeared and the colour of the solution turned yellowish orange. The volume of 0.01N NaOH was noted which corresponds to free acidity. Then 2/3 drops of phenolphthalein was added and titration was continued until a permanent pink colour was developed. The volume of total alkali consumed was noted which corresponds to total acidity. The free acidity and total acidity was

determined using the formula and values are expressed as mEq/L/ 100g.(**P and Palit G.,2001**)

$$\text{Acidity} = \frac{\text{Volume of NaOH X Normality of NaOH X 100}}{0.01} \text{ (mEq/L per 100g)}$$

Ulcer index (UI)

The mucosa was flushed with saline and stomach was pinned on frog board. The lesion in glandular portion was examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined.

- 0 – Normal coloured stomach
- 0.5 – Red colouration
- 1 – Spot ulceration
- 1.5 – Haemorrhagic streak
- 2 – ulcers
- 3 – Perforations

Percentage inhibition

Percentage inhibition was calculated using the following formula.(**Malairajan *et.al.,)***

$$\% \text{ inhibition} = \frac{UI_{\text{ulcer control}} - UI_{\text{ulcer treated}}}{UI_{\text{ulcer control}}} \times 100$$

Statistical Analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison test. The values are statistically significant at three levels, ***$p<0.001$. **$p<0.01$. *$p<0.05$. But ns if $p > 0.05$.

RESULTS AND DISCUSSION

The results of the present study shows that the ethanol extract of ***Excoecaria agallocha linn*** exerts gastro-protective action against ethanol induced ulcer model.

1. PHARMACOGNOSTICAL STUDIES

ANALYTICAL PARAMETERS

The analytical parameters were investigated and reported as, total ash value (9.8%w/w), water soluble ash value (1.5%w/w), acid insoluble ash value (2.8 %w/w),, sulphated ash value (3.5 %w/w), water soluble extractive value (8.8 %w/w), alcohol soluble extractive value (7.5%w/w), loss on drying (4.6%w/w). The above studies were enabled to identify the plant material for future investigation and form an important aspect of drug studies.

A. ASH VALUES

Table 6.1: Ash values parameters.

S.No	PARAMETER	% w/w
	ASH VALUES	
1.	Total Ash	9.8
2.	Water Soluble Ash	1.5
3.	Acid Insoluble Ash	2.8
4.	Sulphated Ash	3.5

B. EXTRACTIVE VALUES

Table 6.2:Extractive values parameters.

S.No	PARAMETER	% w/w
	EXTRACTIVE VALUES	
1.	Water Soluble Extractive	8.8
2.	Alcohol Soluble Extractive	7.5

C. LOSS ON DRYING

Table 6.3: Loss on drying Parameters.

S.No	PARAMETER	% w/w
1.	Loss on Drying	4.6

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Table 6.4: **Fluorescence analysis of the powdered leaves of *Excoecaria agallocha linn***

S.No	POWDER	VISIBLE LIGHT	UNDER UV LIGHT	
			254 nm	366 nm
1	Picric acid	Light brown	Greenish brown	Dark brown
2	Acetic acid	Pale brown	Greenish brown	Brown
3	Conc. Nitric acid	Yellow	Greenish brown	Black
4	Conc. Sulphuric acid	Dark brown	Blackish brown	Black
5	Conc. Hydrochloric acid	Brown	Dark brown	Black
6	Ferric chloride solution	Yellow	Green	Brown
7	Aqueous KOH	Pale brown	Greenish brown	Brown
8	Alcoholic KOH	Pale brown	Green	Dark Green
9	Iodine solution	Brown	Greenish Brown	Dark Brown
10	Ammonia solution 25% v/v	Red	Greenish red	Reddish brown

E. FOAMING INDEX

Table 6.5: Foaming index of the powdered leaves of *Excoecaria agallocha linn*

VOLUME OF ETHANOL EXTRACT (ml)	HEIGHT OF FOAM (cm)
1	2.2
2	3.2
3	3.9
4	3.1
5	4.3
6	5.2
7	5.1
8	5.55
9	5.9
10	5.3

2. PRELIMINARY PHYTOCHEMICAL STUDIES

The leaves of ***Excoecaria agallocha linn*** were subjected for hot continuous extraction using ethanol as solvent and then decoction method using distilled water. The yield for ethanolic extract and aqueous extract was found to be 11.75% w/w. The extracts obtained were subjected to various phytochemical tests and the results were obtained.

Table 6.6: Phytochemical screening of *Excoecaria agallocha linn*

S.NO	PHYTOCHEMICAL CONSTITUNTS	ETHANOLIC EXTRACT
1	Alkaloids	++
2	Saponins	++
3	Tannins	++
4	Terpenoids	++
5	Flavonoids	++
6	Carbohydrates	++
7	Cardiac glycosides	++
8	Phytosteroids	++
9	Amino acids	_ _
10	Gums	++

ANTI-ULCER SCREENING

Ethanol induced ulcer

Effects of ethanol extract of ***Excoecaria agallocha linn*** ulcer index induced by ethanol in rats are shown in Table 1.2

Ethanol induced gastric damage showed gross mucosal lesion, including long haemorrhage bands and petechial lesion. Animals pretreated with ethanol extract of ***Excoecaria agallocha linn*** and standard drug omeprazole showed very mild lesions and sometimes no lesion at all, when compared to ulcer control group.

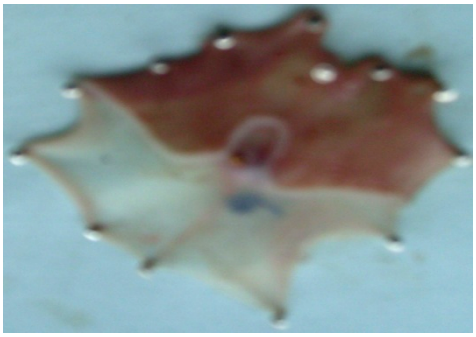
Excoecaria agallocha linn *S*howed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 65.77, 72.54 and 86.59 at doses of 100, 200 and 400mg/kg doses respectively. The

ulcer protective action of extracts at different doses was better than that of standard drug, omeprazole, which exhibited an inhibition percentage of 92.42.

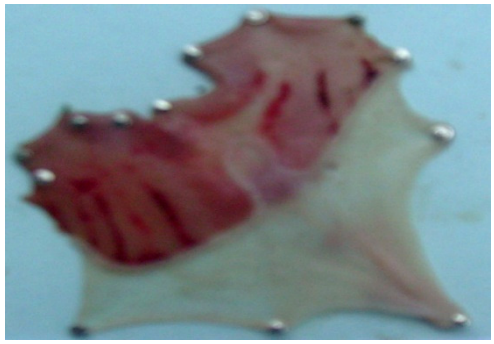
Ethanol produces severe gastric haemorrhagic lesions. the pathogenesis of ethanol induced gastric damage in rats is complicated and involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leucotriene.

Table 6.7: Effect of *Excoecaria agallocha linn* on Ulcer Index in ethanol induced gastric ulcer

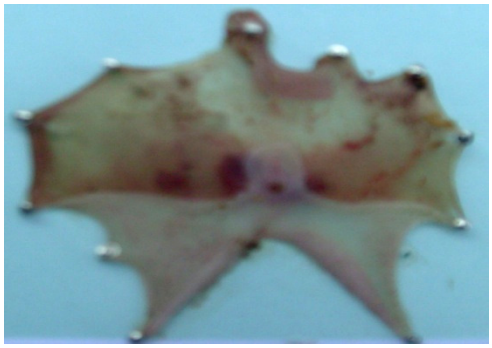
Group	Ulcer index (UI)	Percentage inhibition (%)
Normal Control	00.00 ± 0.00	-
Ulcer Control	17.9 ± 4.76**	-
E.agallocha (100mg/kg)	6.37 ± 1.59*	65.77
***E.agallocha* (200mg/kg)**	5.20 ± 1.31*	72.54
***E.agallocha* (400mg/kg)**	2.57 ± 0.98**	86.59
Sucralfate (100mg/kg)	1.42 ± 0.32**	92.42



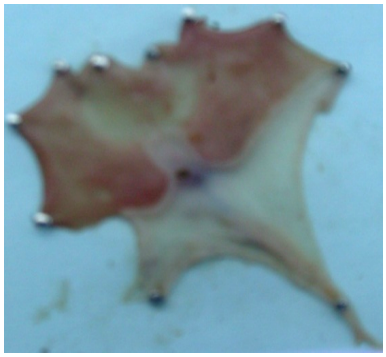
1. Normal Control



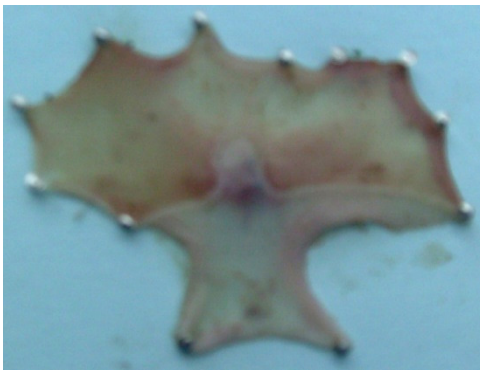
2. Ulcer Control



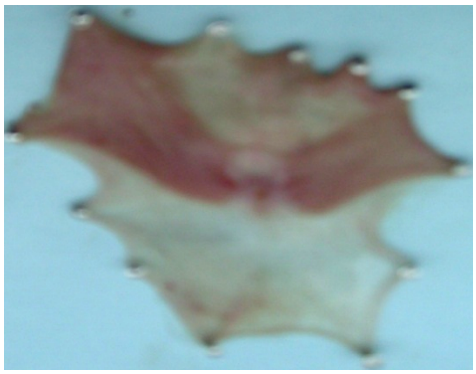
3. *E. agallocha* **(100mg/kg)**



4. *E. agallocha* **(200mg/kg)**



5. *E. agallocha* **(400mg/kg)**



6.omeprazole(100mg/kg)

Fig 6.1: Effect of *Excoecaria agallocha* Linn **on ethanol induced ulcers**

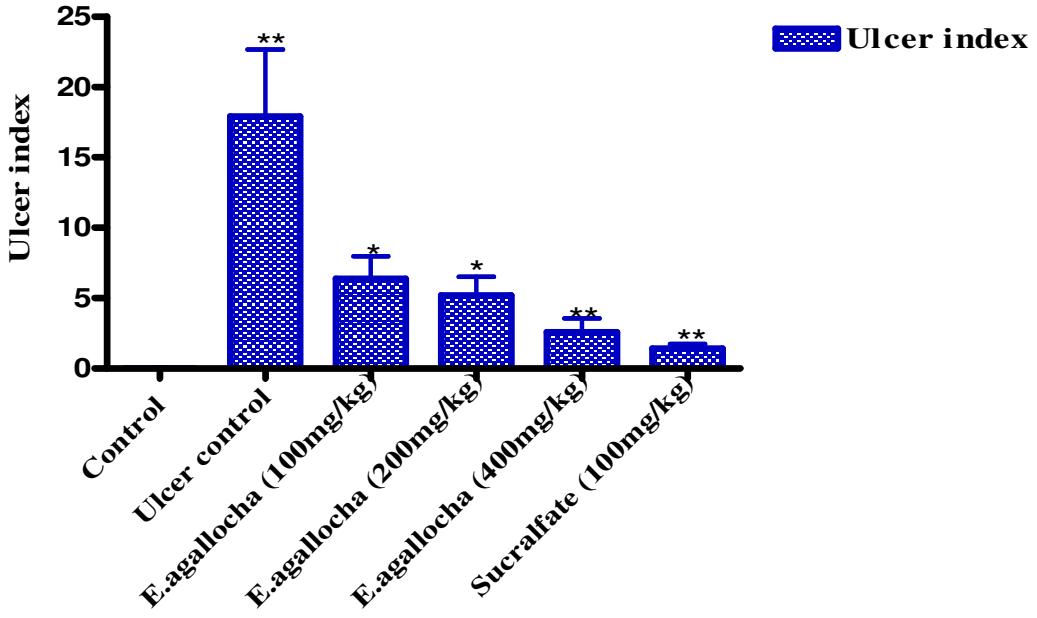


Fig 6.2:Effect of E.agallocha linn on ulcer index in etanol induced gastric ulcer.

Ulcer index (UI) and acid parameters

Oral administration of methanol extract of ***Excoecaria agallocha Linn.*** *at doses of* 100, 200 and 400mg/kg exhibited dose dependent inhibition percentage of 65.77, 72.54 and 86.59 respectively compared to the ulcer control, proving the anti ulcer activity. The standard drug omeprazole (100mg/kg) exhibited a percentage inhibition of 92.42.

The effects of ethanolic extract of ***Excoecaria agallocha Linn***. on acid parameters showed significant ($p<0.001$) effect at 200mg/kg and 400mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH of the gastric juice was increased compared to ulcer control group. But, in this gastric environment also able to induce ulcer, so it can be thought that the antisecretory activity might not be the main mechanism of action of these extracts.

Table 6.8: **Effect of *Excoecaria agallocha Linn.*** on Gastric secretion, total and free acidity using ethanol induced ulcer

Group	Gastric volume (ml/100g)	pH of gastric juice	Total acidity	Free acidity
Normal Control	1.10 ± 0.20	1.89 ± 0.19	77.48 ±3.54	46.30 ±2.76
Ulcer control	3.45 ± 0.19***	1.24 ± 0.15	100.67 ±9.67**	80.33 ±2.45***
***Excoecaria agallocha* Linn. (100mg/kg)**	2.49 ± 0.33	2.01 ± 0.42	52.36 ± 3.43	37.88 ± 1.30***
***Excoecaria agallocha* Linn (200mg/kg)**	1.37 ± 0.10*	1.32 ± 0.10	52.346±3.57***	30.66 ±1.87***
***Excoecaria agallocha* Linn (400mg/kg)**	1.81 ± 0.18***	2.82 ± 0.22**	39.97 ±2.94***	24.63±1.06***
Omeprazole (100mg/kg)	1.33 ± 0.07***	2.79 ± 0.21**	43.96 ±2.03***	25.45 ±0.73***

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. ***P<0.001, **P<0.01, Ulcer control group was compared with Normal control group. omeprazole and Extract treated groups were compared with ulcer control group.

Swimming stress induced ulcer

Oral administration of ethanolic extract of ***Excoecaria agallocha Linn*** 1h before the induction of stress, reduced the water immersion stress induced ulcers. The ethanol extract of ethanolic extract of ***Excoecaria agallocha Linn*** exhibited a dose dependent inhibition percentage of 52.20, 71.16 and 87.79 at doses of 100, 200

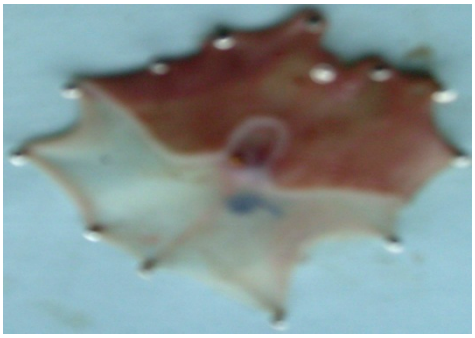
and 400mg/kg dose respectively. The standard drug Omeprazole showed an inhibition percentage of 92.75. The results are shown.

Water immersion stress induced ulcer is one of the best models to induce ulcer in rats. The model provides both emotional stress as well as physiological stress to the animal. Omeprazole was used here to study the proton pump inhibitor mechanism. Water immersion stress induced ulcers are result of autodigestion of gastric mucosal barrier and accumulation of HCl in the stomach.(**Telesphore B.,** *et al*) The methanol extract of *Excoecaria agallocha linn* prevents autodigestion of gastric mucosal barrier in a dose dependent manner in swimming stress induced ulcers due to its cytoprotective action.

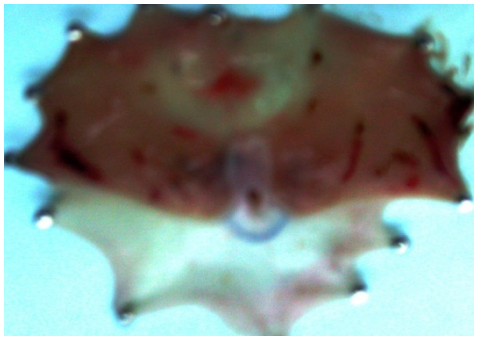
Table 6.9: Effect of Excoecaria agallocha Linn on Ulcer Index in water immersion stress induced gastric ulcer

Group	Ulcer index (UI)	Percentage inhibition (%)
Normal Control	00.00 ± 0.00	-
Ulcer Control	26.66 ± 5.56**	-
***Excoecaria agallocha* Linn (100mg/kg)**	12.45± 1.21*	52.20
***Excoecaria agallocha* Linn(200mg/kg)**	7.70 ± 2.05**	71.16
***Excoecaria agallocha* Linn (400mg/kg)**	3.28 ± 1.76**	87.79
Omeprazole (20mg/kg)	1.97 ± 0.19**	92.75

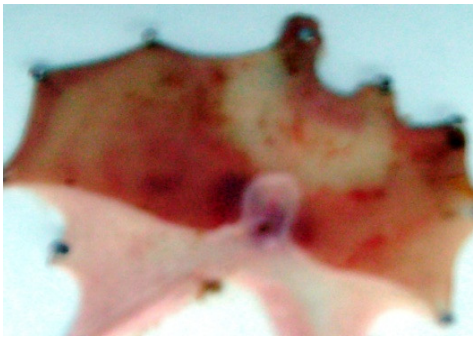
All values are expressed as mean ± S.E.M.; (n=6) animals in each group. **P<0.001, *P<0.01, Ulcer control group was compared with Normal control group. omeprazole and extract treated groups were compared with ulcer control group.



1. Normal Control

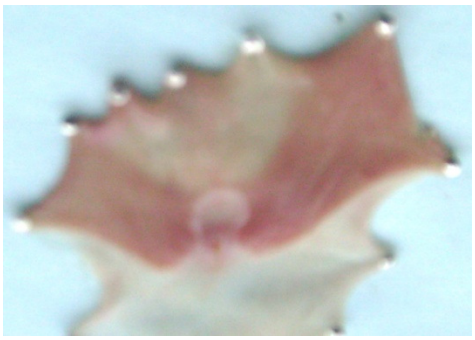


2. Ulcer Control

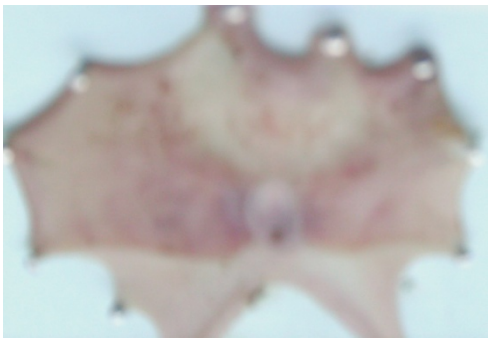


3. *E. agallocha* (100mg/kg)

4. *E. agallocha* (200mg/kg)



5. *E. agallocha* (400mg/kg)



6. Omeprazole (20mg/kg)

Fig 6.3: Effect of *E. agallocha* on stress induced ulcers

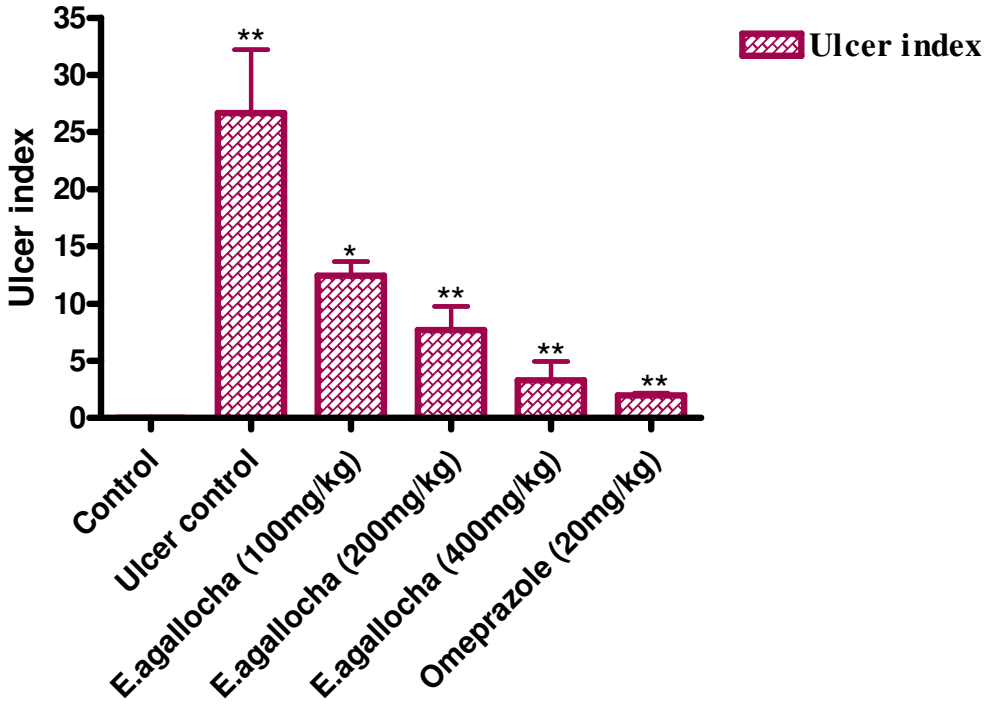


Fig 6.4: Effect of *E. agallocha linn* on Ulcer Index in water immersion stress induced gastric ulcer

SUMMARY AND CONCLUSION

The present study was undertaken to determine the antiulcer activity of ethanolic activity of leaves extract from of ***Excoecaria agallocha linn***.

The pharmacognostical studies made on the leaves extact ***Excoecaria agallocha linn*** ash values, extractive value, loss on drying, fluorescence analysis and foaming index gave valuable information.

The preliminary phytochemical investigation showed the presence of alkaloids, saponins, flavonoids, terpenoids, tannins, cardiac glycosides, gums and phytosteroids.

The pharmacological and acute toxicity studies of ethanol extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed (3 days) up to 2000mg/kg of body weight.

The phytoconstituents like flavonoids, tannins, terpenoids, and saponins have been reported in several anti-ulcer literatures as possible gastroprotective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which antiulcerogenic efficacy has been extensively confirmed.)

It is suggested that these compounds will be able to stimulate mucus, bicarbonate and prostaglandin secretion, and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects.

Excoecaria agallocha linn showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 65.67, 72.43 and 86.49 at doses of 100, 200 and 400mg/kg doses respectively. The ulcer protective action of extracts at 400mg/kg was equal to that of standard drug omeprazole, which exhibited an inhibition percentage of 92.16.

Oral administration of ethanolic extract of ***Excoecaria agallocha linn*** 1h before the induction of stress, reduced the water immersion stress induced ulcers. The methanol extract of ethanolic extract of ***Excoecaria agallocha linn*** exhibited a dose dependent inhibition percentage of 53.30, 71.11 and 87.69 at doses of 100, 200 and 400mg/kg dose respectively. This may be due to proton pump inhibitory activity of the extract. The standard drug omeprazole showed an inhibition percentage of 92.68.

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